DISEASE SYMPTOMS IN *PINGUICULA*: SOME CAUSES AND REMEDIES

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Introduction

Pinguicula species are lovely, little and fragile plants that under good conditions may develop quickly, or frustratingly die just as rapidly for some unknown reason. Even more frustrating is the fact that there is virtually no published information that adequately describes disease symptoms and suggests possible cures. The only disease of *Pinguicula* that has received attention is caused by a filamentous fungus called *Ustilago pinguiculae* (Casper, 1966). This fungus emasculates plants by infecting their pollen. This does not allow the plants to produce seeds and, as a side effect, leads to an enlargement of the vegetative parts. Only some temperate *Pinguicula* are known to be affected.

During the summer of 2000, we observed the recurrence of several diseases in the large collection of Helmut Kibellis. We then subjected several diseased plants to microscopic and microbiological test, and, in the spring and summer of 2001, we investigated several modifications to the growing conditions that appreciably improved the overall health of our collections. This article describes some of the diseases that we encountered and some measures for controlling them. We believe that these diseases are not exclusive to the Sydney region but are found on all major continents.

Materials And Methods

Unless otherwise stated in the text, Mexican *Pinguicula* described in this article were grown in a 1:1:1 mix of perlite, vermiculite, and peat. *Pinguicula lutea* was grown in a 3:1 peat/sand mix. The plants were not fertilized.

The plants of this study were routinely grown by Helmut Kibellis in a glasshouse on a hill in the outskirts of Sydney. The glasshouse was not heated nor cooled and shade was provided by a nearby tree. There was no automatic watering and pots were sitting in trays. The door and some glass panels were opened most of the year. On average, temperatures fluctuated between 18°C to 30°C (64-86°F) in the summer and -1°C to 20°C (30-68°F) in the winter.

Infected plants were transferred to a shade house next to the glasshouse. A 50% shade cloth was used. Temperatures were 2°C to 5°C (1-3°F) lower than in the glasshouse.

Infection and cultivation experiments were conducted on the Hawkesbury campus of the University of Western Sydney. Plants were gown in a polyethylene tent inside a reinforced polyethylene greenhouse. Temperature was lowered by an evaporative cooler and heavy-duty fans. Plants were sprinkled every 5 minutes. Pots were sitting on top of coarse sand to raise air humidity and allow adequate drainage. On average, temperatures fluctuated between 25°C to 35°C (77-95°F) in the summer and 15°C to 25°C (59-77°F) in the winter.

Browning Heart Disease (center-to-edge rosette death)

This is a disease that causes one of the most spectacular, sudden deaths of *Pinguicula* plants. It is characterized by the browning (dark brown/black) and death of the center of the

Figure 1: Browning heart disease in *Pinguicula rectifolia* plant. The dead rosette center is infected by nematodes and *Fusarium*.

Figure 2: Browning heart disease in *Pinguicula rectifolia* plant at 10X magnification. The dead leaf shows *Fusarium* hyphae emerging from the dead zone.

Figure 3: *Fusarium* spores. Spores were extracted from the soil that surrounded a browning heart diseased plant, stained with cotton blue for enhanced contrast and examined with a light microscope at 400X magnification. *Fusarium* spores exhibit a typical sickle shape and cell wall boundaries between cells.

rosette that rapidly (in a couple days) spreads to the tips of all of the leaves. This symptom is most often seen on central American *Pinguicula* during the spring or hot summer days and has a tendency to be epidemic in a collection. It is particularly severe in the Sydney region because of the hot and dry springs/summers (especially the period of three months from October to December). We refer to it as browning heart disease. Early signs of this disease include a loss of vigor and no new leaf growth (even though spring should witness the abundant production of fresh new growth). Plants are stagnant with non-mucilaginous, dull green leaves. There is no development of young white roots and, if the plants had them when they were healthy, the roots turn brown, shrivel and die. The rootless base of the plant becomes a black stump that looks like a tap root—typically an 8 cm (3 inch) diameter plant will produce a 1 cm (0.4 inch) long black root stump. The base of the leaf petioles then turn rusty-brown before the center of the rosette blackens and the whole plant dies.

During each of two separate outbreaks of browning heart disease that we observed, three sick *P. rectifolia* plants were sampled (i.e. a total of six plants). The tips of the leaves of these plants were still green while their bases were brown (Figure 1). Microscopic observation revealed the presence of numerous fungal hyphae entering and leaving the brown area (Figure 2). Depigmentation and softening of the tissues in a zone between the brown and healthy areas could also be seen—these effects are typical of spreading fungal infections. Tissue samples (1×1 mm) taken from the brown area of three separate leaves of each of the diseased plants were plated on sterile potato dextrose agar medium. In each case, a similar white mycelium grew rapidly from the sampled tissue. After about two months, conidia developed and were examined under the microscope for identification. The fungi invariably belonged to the genus *Fusarium* (Figure 3).

All of the plants under study were gently unearthed. A sample of soil from just under the center of the rosettes was mixed with a drop of sterile water and examined under the microscope. Vast numbers of *Fusarium* spores and nematodes were observed. Male and female nematodes could be seen (Figure 4). The presence of nematode damage near the base of the dead roots was also visible. On a live root, each point of damage from the nematode looked like a swollen brown

Figure 4: Nematodes extracted from the soil that surrounded a browning heart diseased plant, visualized with a field contrast microscope at 400X magnification. The photo shows a large female at the center and two smaller males around her.

dot. If peat was used in the potting mix, tiny peat particles adhered to the wounds after rinsing the roots under a stream of water.

Several cuttings made from the green portions of half-dead leaves took root, therefore suggesting that the leaves did not suffer from any internal physiological disorder.

The above observations suggest that the browning heart disease in Mexican *Pinguicula* is associated with a combination of *Fusarium* and nematode attack. Such a combination is lethal to numerous plant species. Female nematodes will enter plant roots to feed. This leads to a weakening of the plants. *Fusarium* spores and hyphae naturally present in the soil are normally unable to penetrate intact tissues but will enter the wounds made by the nematodes. From there, the fungus will quickly develop through the plant to lead to its death. Interestingly, my experiences in plant pathology have shown me that central and south American plants (such as cacti, tomato, potato, etc.) are more sensitive than other plants to such a combination of *Fusarium* and nematode attack.

Rosette Edge-to-Center Death

A different sudden death syndrome plagues *Pinguicula* species from the southeastern USA. In this case, the symptoms appear in late winter/early spring when the temperature is raised too rapidly. It is characterized by a browning of the tip of the leaves that spreads towards the center of the rosette. It will be referred to as rosette edge-to-center death. Tests for the presence of microbial contaminants have been conducted on dead parts as described above. However, no pathogen could be extracted from the brown areas. Therefore, this symptom, which occurs on winter-resting plants during rapid elevations of temperature, most likely stems from an internal metabolic imbalance resulting from a differential acceleration of the various metabolic pathways during temperature rises. Enzymatic systems work as a chain factory so that the greater excitation of some elements by increased temperature over others will lead to the accumulation in the tissues of the plant of intermediate toxic metabolites that can lead to self-poisoning. This explanation is compatible with the observation that, once induced, the dying back never stops even if the necrotic area is cut off (the plant has actually poisoned itself completely during a temperature shock and we are witnessing the differential spread of senescence of its tissues). Slower elevations of temperature (as are more common in outside settings like in natural environments than in glasshouses) allow survival since they provide more time for the plants to re-equilibrate their enzymatic activities and adapt to higher temperatures.

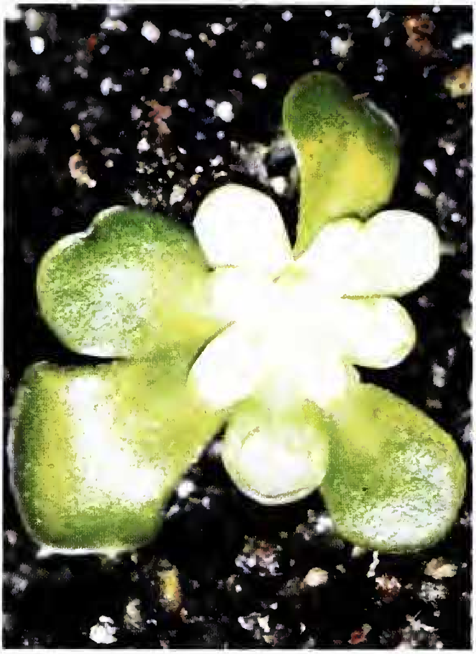

Figure 5: Whitening of the center of a *P. rectifolia* plant due to mineral deficiency.

Figure 6: A *Pinguicula lutea* plant with white leaf symptom.

Figure 7: A cross-section of a white *P. lutea* leaf was examined with a light microscope (12X). Most cells appear white. Only a few cells located close to the tracheid elements (center of section) and on the leaf underside contained chloroplasts (small green balls).

A less serious disease involves the melting (total disappearance) of leaves as they touch the soil. The breakdown of the tissue often starts at the spot where the leaves touch the soil. A round hole develops in the middle of the leaf lamina that expands to the rest of the leaf in a few days. The formation of such holes may lead to the death of a whole leaf but rarely results in the death of the whole plant. Microbiological tests conducted on the dead tissues revealed the presence of either of two types of filamentous fungi, *Botrytis cinerea* or *Trichoderma* spp. These two types of fungi are necrotrophic pathogens that normally live upon dead plant parts. *Trichoderma* species are rarely pathogenic tó plants (in fact, they often act as biological control agents by preventing the development of other pathogenic fungi). When grown on a Petri dish, the *Trichoderma* spp. extracted from the *Pinguicula* leaves exuded an attractive coconut smell. The *Botrytis* isolates that were extracted proved to be incapable of infecting healthy *Pinguicula* plants when added onto their leaves as conidia or mycelial plugs. Therefore, it seems likely that these infection holes may be the result of potassium/calcium deficiencies in the plant since in many horticultural crops such deficiencies lead to greater sensitivity to microbial attack by weak pathogens (Resh, 1995).

To test this hypothesis, *Pinguicula* of several species (1 cm rosette diameter, from tissue culture) were transplanted into large polystyrene foam boxes filled with standard peat/sand media. Both a control mix and a richer mix (Mix A, derived from a University of California, Davis hydroponic mix; see Resh (1995) for details) were used.

The lamina hole symptoms did not appear on plants grown in Mix A and older leaves survived much longer. In some instances it led to the production of rosettes bearing a greater number of leaves and the stacking of several layers of green leaves. The plants in general looked healthier, grew faster, and in some instances, bloomed more and generated more seeds. The addition of extra nitrogen to the mix (Mix B—Table 1) led to no further stimulation in plant growth but allowed the resumption of lamina hole formation.

After three months of growth, the rosette diameters were measured. Flower production was monitored for six months after initial insertion of the plants into the media. The results are given in Table 2. We believe the resumption of lamina hole formation was because high nitrogen fertilization typically leads to increased sensitivity to microbial attack and to the greater development of microbes in the soil (Resh, 1995).

Table 1: Composition of potting mixes

Component	Control Mix	Mix A	Mix B
peat moss[1]	5 kg (=1V)	5 kg (=1V)	5 kg (=1V)
sand[2] (1 mm quartz)	1/2 V	1/2 V	1/2 V
potassium nitrate	—	11.34 g	11.34 g
potassium sulfate	—	11.34 g	11.34 g
superphosphate	—	113.6 g	113.6 g
dolomite lime	—	341 g	341 g
calcium carbonate lime	—	113.6 g	113.6 g
trace elements	—	3.865 g	3.865 g
blood and bone	—	—	113.6 g

[1]Peat moss originated from Lithiania.

[2]The base of these mixes is made by combining two parts (by volume) peat to 1 part sand.

Table 2: Growth characteristics of *Pinguicula* species on two potting mixes.				
Species	Control mix		Mix A	
	Rosette diameter[1] (cm)	Flowers presence	Rosette diameter[1] (cm)	Flowers presence
P. agnata	2.5	No	8.2	Yes
P. filifolia	4.3	No	5.3	No
P. crystallina subsp hirtiflora	2	Scarce	3.75	Yes
P. emarginata	2.3	No	4	Yes
P. lusitanica	1.7	Yes	1.7	Yes
P. moranensis	5	No	5	Yes
P. primuliflora	1.7	No	2.6	No
P. sp.—Ayautla[2]	2.5	No	24	Yes
P. sp.—Huahuapan[2]	1.5	No	2.3	No
P. sp.—La Vuelta[2]	4	No	4.2	No
P. sp.—Pachuca[2]	4	No	5.8	Yes
P. sp.—Synalta[2]	9	No	21	Yes

[1]Rosette diameters were recorded three months later and flower formation over a period of six months. Values represent the average of recordings made on at least three plants. Standard deviations were less than 10% of the mean.

[2]The identification of this plant is unknown. The name after the dash indicates Mexican provenance information.

Leaf Whitening

Another minor disease symptom involves the whitening of old or young leaves (Figure 5). These symptoms are common, and, generally speaking, *Pinguicula* plants tend to look whiter in most collections than they do in their native habitat. As for most plants, this may result from a deficiency in various microelements such as zinc, boron or iron (Resh, 1995). In agreement with this hypothesis, growing some *Pinguicula* species in Mix A or Mix B led to the re-greening of their leaves within three months.

There is another form of leaf whitening that is specific to *P. lutea*. In the summer, the central section of the newly developing leaves turn pure white while the tips and bases remain green (Figure 6). Microscopic examination of sliced white parts did not reveal any damage caused by insects, mites or thrips. The cells lacked chloroplasts but looked healthy (Figure 7). The most likely cause of this symptom is an excess of light since a plant growing under shade redeveloped green leaves.

Discussion

Our study reveals that disease symptoms in *Pinguicula* are not always linked to microbial attacks. Temperature shocks, light induced bleaching and mineral deficiencies can all generate physiological disorders, and in the case of temperature variations, may lead to death. Mineral deficiencies are particularly common in hobbyists' collections because of the widespread use of perlite/vermiculite mixes and no fertilization. These are hard to spot just after repotting and may take several months to fully develop. A transient improvement in plant health may even appear at first because of the washing of excess salts from a previous mix or sudden improved aeration. However, the nutritional needs of *Pinguicula* and their relationship with temperature, soil pH and texture are unknown. Our study also suggests that one mix may not fit the needs of all species (see the different reactions of different species to Mix A) and preliminary results showed that young plants tend to prefer a poorer mix than their adult forms. More work is required in this area.

The most severe microbe-related disease we found was linked to the penetration of *Fusarium* spp. into plant roots via wounds made by nematodes. Even though both protagonists need to be present simultaneously to kill the plants, their ubiquitous nature in the air and water respectively makes this deadly cocktail a common occurrence feared by most nurserymen. There is no obvious cure for this. Most nematicides greatly reduce nematode colonies but never eradicate them (e.g. Nemacur 400® - 0.1% fenamiphos) so that within three weeks after treatment, their ranks regrow. Similarly, anti-*Fusarium* fungicides (we tried Bayleton® from Bayer) only stop momentarily the development of the fungus (these substances should be called fungistatics rather than fungicides), as its growth will resume about two weeks later. Since the movement of air-borne spores of *Fusarium* species is hard to prevent, the focus of any cure is usually to prevent nematode entry into the collection. Nematodes are carried via contaminated water, utensils and soil pieces attached to shoes. Nematodes are, however, very sensitive to heat (hot water or steam). We, now, routinely heat-sterilize our potting mixes (for example, in a Chinese crock-pot instrument). Instruments and pots are soaked in bleach prior to use. The use of large trays or shared cascade water is dangerous because any contaminated pot will infect its neighbors. Occasional outbreaks are unavoidable and infected plants should be quarantined swiftly. Our only success in saving infected plants has been obtained after moving the affected plants away from the healthy ones from their original glass-house to a separate shade-house (50% shade cover) to improve ventilation. The plants were kept totally dry and after several weeks, new growing points could be seen at the base of the petioles (like in leaf cuttings) and at the center of the sick rosette. At that stage, the plants were potted into sterilized pots and potting mix.

The new use of overhead sprinklers that automatically (timer-controlled) water our plants briefly a couple times per day (9 a.m. and 4:30 p.m.) has greatly helped in many ways. First, it replaced the use of trays, which act as a pathway for nematodes to spread from pot to pot. Second, it prevents the accumulation of abundant water-borne salts while providing access to larger quantities of trace minerals to the plants. Third, by using overhead sprinklers, a larger volume of water (and its minerals) flows past the roots compared to the tray system. The plants are, therefore, less likely to exhibit mineral deficiency symptoms. Fourth, overhead watering also cools the plants and their potting mixes and therefore prevents sun-induced heat damage in closed glasshouses. Finally, the passage of a layer of water from the top to the bottom of the mix keeps the water in the soil oxygenated. It brings fresh oxygen to the roots and removes the CO_2 the roots have been producing via their own respiration.

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